Transcriptomic profiling of clobetasol propionate-induced immunosuppression during a TLR-7-dependent immune challenge in zebrafish embryos

B. Luckner[1,2], F. Essfeld[2,3], S. U. Ayobahan[2], E. Eilebrecht[4] , E. Richling[5] and S. Eilebrecht[2]
[1] Toxicology, Faculty of Chemistry, RPTU Kaiserslautern-Landau, Germany
[2] Fraunhofer Attract Eco'n'OMICs, Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Schmallenberg, Germany
[3] Computational Biology, Faculty of Biology, Bielefeld University, Bielefeld, Germany
[4] Department Ecotoxicology, Fraunhofer IME, Schmallenberg, Germany
[5] Food Chemistry and Toxicology, Department of Chemistry, RPTU Kaiserslautern-Landau, Germany

Background

Due to the lack of standardised test methods and validated biomarkers, chemical-induced immunotoxic modes of action (MoA) currently remain unconsidered in the regulatory environmental hazard assessment. Via transcriptomic analysis of chemically immuno-suppressed and/or immune-challenge zebrafish embryos (ZE), this study provides an approach to evaluate postulated and identify novel biomarker candidates for immunotoxic MoA. Additionally, this study aimed to assess the suitability of the ZE as an alternative system for the imiquimod (IMQ)-induced psoriatic mouse model. With this we want to contribute to the development of reliable adverse outcome pathways, to predict the immunotoxic potentials of chemicals in the future.

Methods

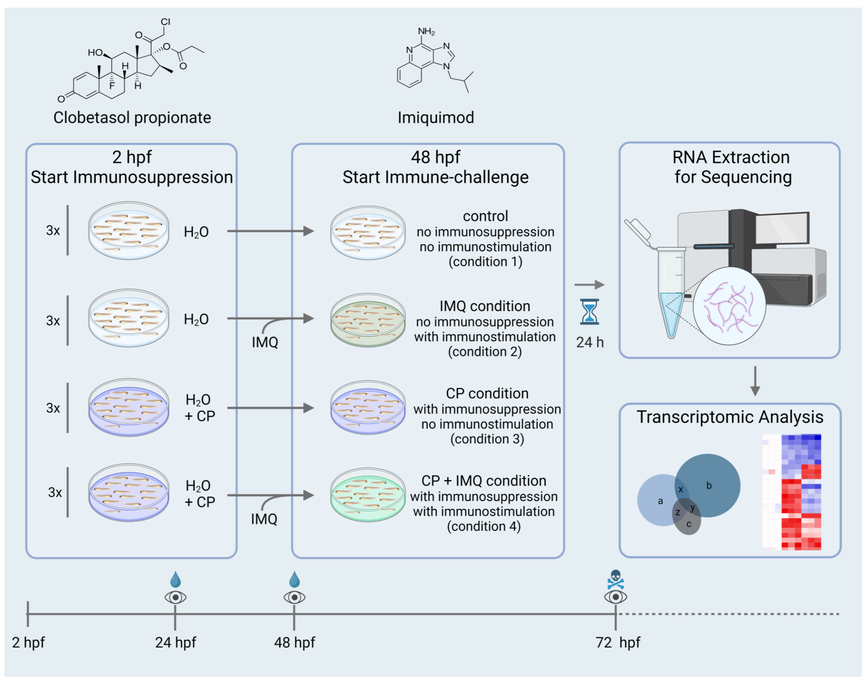

__Figure 1__: Experimental workflow with a detailed view on defined conditions. hpf = hours post fertilization. Eye: Microscopical inspection. Drop: Medium exchange. Skull: Euthanasia. Created with BioRender.

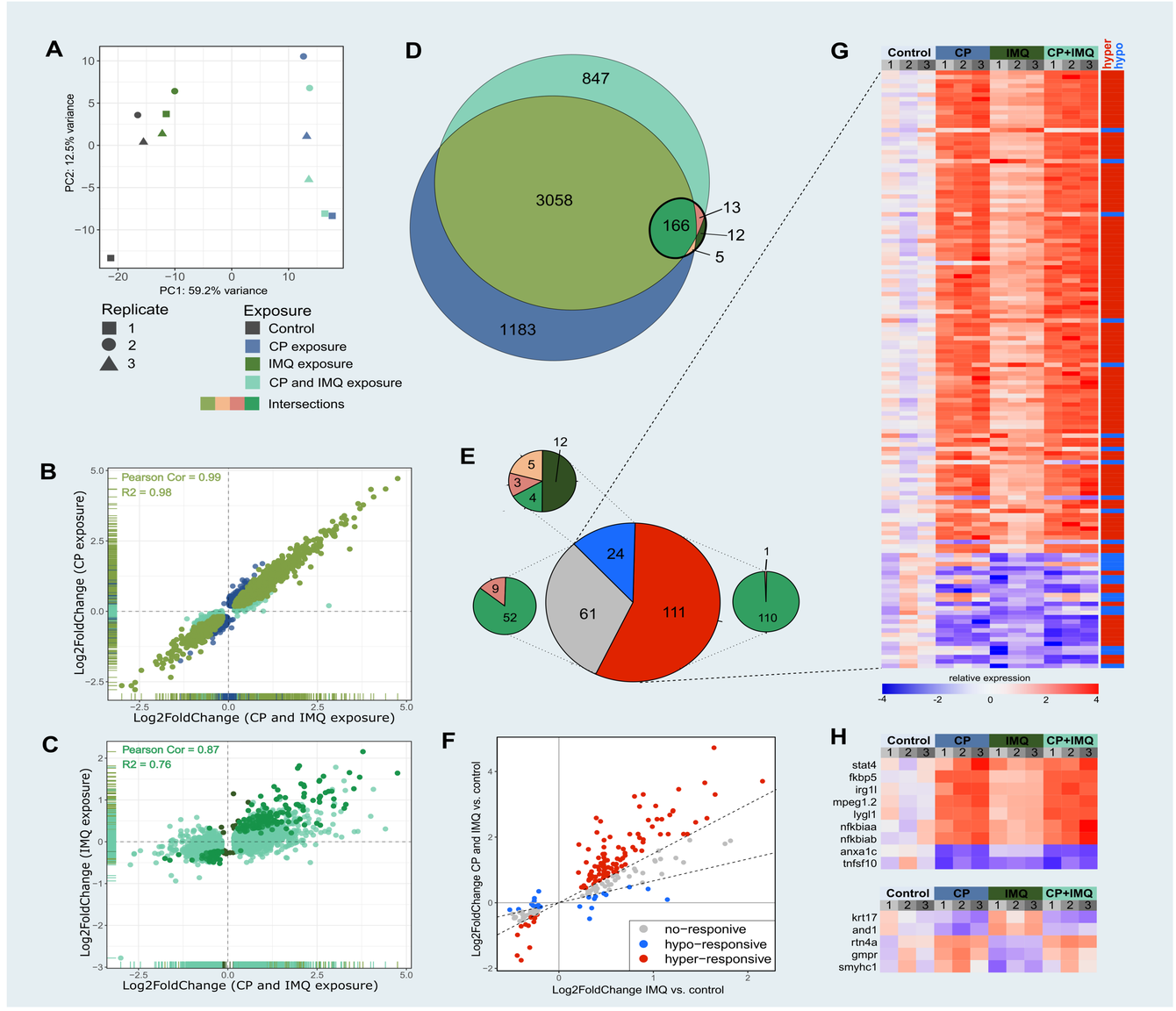

__Figure 2: A__: PCA plot. __B+C__: Scatterplot comparing log2fold change (LFC) values of common DEGs between the single and combined treatment. __D:__ Total number of detected DEGs in all conditions. __E:__ Identification of IMQ-responsive DEGs in hyper, hypo-, non-responsive and their affiliation related to D. __F:__ Scatter plot comparing LFC values of hyper-, hypo-, non-responsive genes between IMQ and CP+IMQ condition. __G__: Heatmap showing the relative expression of hyper- and hypo-responsive genes in all conditions. __H__: Examples of detected previously published immune-related genes (top) and novel candidates for immunotoxic biomarkers (bottom).

Results and Discussion

Revealing a total variance of 71.7 % and a clear clustering of conditions, the principle component analysis (PCA) (Fig. 2 A) confirmed that transcriptomic profiles of all conditions differed from the control, while the CP treatment has had a stronger impact than the IMQ treatment. The Venn diagram (Fig. 2 D) shows the total number of detected DEGs for all conditions. Intersections show sets of common DEGs between the conditions. The scatter plot (Fig. 2 B) shows that most detected DEGs where regulated similarly in response to the CP and the CP+IMQ condition. In contrast, although still highly correlated, less similarity was observed in the regulation comparing the IMQ and the CP+IMQ treatment (Fig. 2 C). This indicates that the prior immunosuppression was capable to effectively alter the immune-response of the organism. To analyse the extent of this effect in further detail, IMQ-responsive DEGs were characterised to be either hyper-, hypo-, or non-responsive. Hyperresponsiveness was defined as showing a > 1.5 times higher LFC value and hyporesponsiveness as showing a > 1.5 times lower LFC value in the combined CP+IMQ vs. the single IMQ treatment, indicating synergistic / agonistic effects of CP and IMQ on these genes. The pie charts in Fig. 2 E show the number of characterised DEGs and their affiliation in respect to Fig. 2 D. A comparison of the LFC values of DEGs between the single IMQ and combined treatment is depicted in Fig. 2 F. These findings confirm that environmental contaminants are capable to impact or even perturb the defence response of an organism which is of crucial importance for its survival, highlighting the necessity to assess the immunotoxic potential of chemicals. Looking closer at the data, detected previously proposed immunotoxic biomarkers, such as *fkbp5, socs3, mpeg1.2, nfkbia, anxa1c, irg1l, stat4* were re-evaluated[1,2,]. Data showed that these genes were regulated in the same direction in response to both tested compounds (Fig. 2 H, top). This substantiates their usability as potential biomarkers for immunotoxic MoA, likewise, however, unmasks them as unsuitable to differentiate between a suppressive / stimulatory fashion of effect. The dataset was scanned for genes, regulated in opposite directions by both compounds revealing *krt17, and1, rtn4a, gmpr* and *smyhc1* as novel immunotoxic biomarker candidates with said power to differentiate. The observed IMQ-induced regulation of gene expression in a pro-inflammatory manner and the induction of a known alarmin for psoriasis (*krt17*)[3] substantiates the usability of the ZE as an alternative system for the IMQ-induced psoriatic mouse model.

Conclusion

Without limiting the analytical view, but rather allowing to analyse the global level of e.g. gene expression, this study shows that OMICs techniques provide a powerful tool for the identification of novel biomarkers and compound-specific molecular signatures. These approaches and the identified biomarker candidates will help to develop reliable testing methods to address immunotoxicity in the environmental hazard assessment in the future.

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1 Essfeld, Fabian, et al. "Transcriptomic profiling of clobetasol propionate-induced immunosuppression in challenged zebrafish embryos." Ecotoxicology and Environmental Safety 233 (2022): 113346.
2 Willi, Raffael Alois, et al. "Active glucocorticoids have a range of important adverse developmental and physiological effects on developing zebrafish embryos." Environmental science & technology 52.2 (2018): 877-885.
3 Leigh, I. M., et al. "Keratins (Kl6 and Kl7) as markers of keratinocyte hyperproliferation in psoriasis in vivo and in vitro." British Journal of Dermatology 133.4 (1995): 501-511.